FGFR signalling promotes the growth of triple negative and basal-like breast cancer cell lines both *in vitro* and *in vivo*

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Supplementary material

Supplementary tables

Supplementary table 1. Array CGH of PD173074 sensitive basal-like cell lines.

Gene	Chromosme	Start (bp)	MFM223	SUM52	CAL120	HCC1143	CAL51	BT549	HS578T	MDA-MB-157	MDAMB453
FGF1	5	141953305	-0.0772	0.2515	0.1701	0.0901	-0.0237	0.2399	0.0449	0.4343	-0.0375
FGF2	4	123967312	0.018	-0.1121	0.0089	-0.1594	-0.0203	-0.0311	-0.0706	-0.0363	0.0167
FGF3	11	69333916	**1.0036**	0.1752	0.0872	**0.9494**	0.0102	-0.0148	0.0584	-0.115	0.2023
FGF4	11	69296977	**1.0036**	0.1752	0.0872	**0.9494**	0.0102	-0.0148	0.0584	-0.115	0.2023
FGF5	4	81406765	-0.2332	-0.1121	0.0089	-0.1594	0.022	-0.0311	-0.0706	-0.0363	0.0199
FGF6	12	4413568	0.0749	-0.0833	0.3025	-0.1633	-0.1094	-0.1387	-0.1446	-0.4505	-0.1127
FGF7	15	47502750	0.2465	0.0894	-0.1579	0.0578	0.0439	-0.0373	-0.0046	0.0602	0.3904
FGF8	10	103519876	0.0599	-0.0725	-0.1906	-0.1001	0.0361	0.0057	-0.1456	-0.2251	-0.0287
FGF9	13	21143874	-0.1864	-0.131	-0.1088	-0.0257	0.0369	0.09	-0.0999	-0.2678	-0.0197
FGF10	5	44340853	**0.5728**	0.2193	**0.5647**	0.0886	0.0307	0.166	0.2591	0.2071	0.0045
FGF16	23	76596302	-0.1774	0.0471	-0.1011	0.1225	-0.0238	-0.1733	-0.2421	0.1004	-0.194
FGF17	8	21956373	-0.3411	-0.3134	-0.2903	-0.3606	6.00E-04	0.2681	0.123	-0.1531	-0.4743
FGF18	5	170779271	-0.0958	0.192	0.155	0.2189	0.0191	0.1391	0.0465	0.264	-0.0534
FGF19	11	69222186	**1.0036**	0.1752	0.0872	**0.9494**	0.0102	-0.0148	0.0584	-0.115	0.2023
FGF20	8	16894704	-0.1819	-0.2897	-0.1405	-0.2194	-0.0726	0.3942	0.2666	-0.0479	-0.4485
FGF21	19	53951155	-0.2463	0.4252	-0.0209	-0.0316	0.0682	-0.1739	-0.0193	-0.2348	-0.0431
FGF22	19	590925	0.0313	0.1848	-0.0515	-0.0739	0.0044	-0.1567	-0.0283	-0.1269	0.071
FGF23	12	4347653	0.0749	-0.0833	0.3025	-0.1633	-0.1094	-0.1387	-0.1446	-0.4505	-0.1127
FGFR1	8	38387812	0.6009	0.7143	**0.9978**	-0.0669	6.00E-04	0.3562	0.2327	0.2879	-0.0358
FGFR2	10	123227833	**2.3466**	**2.8187**	-0.1906	-0.1439	-0.037	-0.2036	-0.003	0.0045	-0.0287
FGFR3	4	1764836	0.0789	0.076	0.2347	-0.1213	0.0095	-0.0891	-0.1098	-0.304	-0.1099
FGFR4	5	176446526	0.2127	0.2991	0.1021	0.2189	0.0685	0.0609	-0.0177	0.264	-0.0534
FGFRL1	4	995609	0.0789	0.258	0.09755	-0.1213	0.2344	-0.0891	-0.1098	-0.0851	-0.1099

Array CGH CBS smoothed log2 ratios of *FGFR* and *FGF* genes in cell lines with sensitivity to PD173074 (Figure1). Where more than on BAC overlies the gene of interest the median value is displayed. Black indicates amplified genes (log2 ratio > 0.45). HCC1143 and MFM223 displayed amplification of *FGF3*, *FGF4* and *FGF19*, which are adjacent to *CCND1* on chromosome 11q, but did not express the genes.

Supplementary table 2. Gene expression Illumina WG6 V2 array data for *FGFR* and *FGF* genes in breast cancer cell lines.

Probe	Gene	MDAMB134	ZR75.1	S68	SUM44	VP229	MFM223	SUM52
ILMN_28534	FGF1	6.60108773	6.61878205	6.62495778	6.6156604	6.59866027	6.64930554	6.62150334
ILMN_28534	FGF1	6.64537655	6.6301744	6.66538145	6.64837507	6.62674637	6.64649349	6.74979337
ILMN_20787	FGF2	6.64668285	6.64288121	6.69304254	6.74289808	6.75141835	6.83015934	6.6610835
ILMN_27066	FGF3	6.76538699	6.90392531	6.80990693	7.00810865	6.85287606	7.30457923	6.90960075
ILMN_12573	FGF4	6.65137491	6.6841231	6.69515783	6.63512584	6.64160893	6.6852132	6.64729413
ILMN_12488	FGF5	6.63238897	6.61338219	6.62742448	6.64359733	6.64010454	6.64247034	6.69072069
ILMN_12829	FGF5	6.65950163	6.64155449	6.66650594	6.64206921	6.69846947	6.65018676	6.63387542
ILMN_28260	FGF6	7.28664991	7.54323492	7.40165098	7.32262939	7.26187225	7.40212227	7.28599762
ILMN_20000	FGF7	6.5448421	6.6312652	6.60091867	6.62883272	6.64722961	6.64045385	6.62281722
ILMN_1423	FGF8	6.64469698	6.71708784	6.7369951	6.66053651	6.6445981	6.76336143	6.6648293
ILMN_1771	FGF9	7.15174977	6.60264833	6.62387921	6.60763586	6.60269185	6.55324635	6.61191768
ILMN_19131	FGF10	6.73292207	6.64610748	6.6433161	6.64657361	6.65295543	6.66495044	6.64747075
ILMN_22755	FGF16	6.63380503	6.61492051	6.6251714	6.64274942	6.63238986	6.56811618	6.63533734
ILMN_9431	FGF17	6.93942952	6.76754458	6.98202343	6.7046626	6.89925873	6.87316086	7.04765702
ILMN_29689	FGF18	7.58060538	7.45963385	7.35779421	7.54421447	7.55957043	7.20582996	7.51336953
ILMN_8856	FGF18	7.33797473	7.3380542	7.33369832	7.5394021	7.3822405	7.4741057	7.73064421
ILMN_18897	FGF19	6.97859632	6.99005725	7.00987745	7.10122992	7.09559723	6.92388225	6.9589594
ILMN_10954	FGF20	6.7978987	6.88179978	6.81584489	6.87672092	6.70703408	6.86507631	7.29989838
ILMN_9969	FGF21	6.65694616	6.64743329	6.6507929	6.62099805	6.65666576	6.66666102	6.81546161
ILMN_15552	FGF22	6.75032038	6.80885217	6.66271623	6.70668389	6.68192701	6.64114235	6.89084188
ILMN_10473	FGF23	6.71181709	6.64210799	6.66166503	6.6936163	6.72769781	6.7152618	6.69283086
ILMN_7476	FGFR1	9.35649007	7.17041639	8.21439848	8.10607313	6.81333707	7.09393512	6.68461488
ILMN_168227	FGFR2	6.57835576	6.61930396	6.63762517	6.50667029	6.62273436	12.2761235	12.0025256
ILMN_22960	FGFR3	6.76205725	6.90112253	6.76413665	6.64884108	6.82117035	6.75472841	6.85232472
ILMN_22960	FGFR3	8.69654743	10.375919	7.96317285	8.87355338	10.8806229	7.74217283	7.36445369
ILMN_29492	FGFR4	9.19335902	7.92275235	7.03125153	9.73172715	6.64427087	8.86858321	9.01074643
ILMN_28273	FGFRL1	10.1525596	10.2444954	8.62250185	9.5589409	9.78284332	8.80537005	9.04446609

Probe	Gene	CAMA1	CAL120	HCC1143	CAL51	MCF7	JIMT1	SKBR3	BT474
ILMN_28534	FGF1	6.59947191	7.84578001	6.66231077	6.61976226	6.65147821	6.63191593	6.65203229	6.67472686
ILMN_28534	FGF1	6.70412761	8.50346244	6.75705324	6.70113782	6.75182079	6.71742548	6.80150958	6.77161574
ILMN_20787	FGF2	6.69115413	7.5792541	6.70495668	7.01223588	6.64466466	6.68591328	6.65763941	6.66499343
ILMN_27066	FGF3	6.74467399	6.90831963	6.74521182	6.84088603	6.96620411	6.75323098	6.83024866	6.72428334
ILMN_12573	FGF4	6.6454075	6.67228394	6.69947678	6.78793351	6.61006113	6.70602885	6.86270071	6.66454707
ILMN_12488	FGF5	6.6819703	7.70210391	6.72065042	6.65850401	6.63143795	6.68867909	6.68142007	6.66108747
ILMN_12829	FGF5	6.6838121	8.3486691	6.62845418	6.64427126	6.63349085	6.67557173	6.72742332	6.69698734
ILMN_28260	FGF6	7.26864678	7.22623718	7.06478961	7.26532845	7.14008789	7.18533223	7.29141469	7.20867839
ILMN_20000	FGF7	6.62850712	6.62152832	6.59942576	6.62260536	6.61923401	6.63785778	6.62173756	6.59476453
ILMN_1423	FGF8	6.80268808	6.70318417	6.66249632	7.50983127	6.66773991	6.72015262	6.67106524	6.72069394
ILMN_1771	FGF9	6.62488993	6.61889155	6.66159825	6.62210674	6.65081714	6.60862918	6.6417191	6.6532869
ILMN_19131	FGF10	6.75482875	6.64867372	6.70217637	6.67258333	6.66838774	6.64709776	6.68322435	6.64935752
ILMN_22755	FGF16	6.62566856	6.67657633	6.61475632	6.66814202	6.63658146	6.6555462	6.63581361	6.64678824
ILMN_9431	FGF17	7.02242788	7.12288688	6.71572989	6.8710981	6.82371687	6.99532344	6.89565924	6.84285588
ILMN_29689	FGF18	7.73681521	7.70957157	6.81904309	10.1439996	7.04807605	7.68832241	7.2750785	7.24099964
ILMN_8856	FGF18	7.71037183	7.63971568	7.19327152	7.26846268	7.25221972	7.49658799	7.63700414	7.55283654
ILMN_18897	FGF19	7.16051598	6.98264208	7.05502157	6.86925998	6.85506848	6.95828347	6.83804011	6.93319759
ILMN_10954	FGF20	7.10120536	7.24405087	6.71563182	6.74151064	6.68687678	6.79576609	7.01284409	6.84372334
ILMN_9969	FGF21	6.71689108	6.64954993	6.83262815	6.65228692	6.65995145	6.66515898	6.68835855	6.66111356
ILMN_15552	FGF22	6.68253619	6.64295488	6.71069123	6.74225433	6.67280508	6.65904089	6.66575271	6.67901923
ILMN_10473	FGF23	6.74661401	6.78682226	6.79495912	6.94976042	6.66683614	6.74190488	6.90098066	6.89059776
ILMN_7476	FGFR1	6.83574114	9.40862218	6.86524114	7.13188257	6.84313454	7.20869496	6.6599717	6.68170143
ILMN_168227	FGFR2	6.64161391	6.62989551	6.67718545	7.94573774	6.83989166	6.6390849	6.81176197	6.64900158
ILMN_22960	FGFR3	6.790077	6.8485189	6.74216606	6.8395155	6.81961987	6.6390849	6.78104739	6.69336955
ILMN_22960	FGFR3	8.20747949	7.4667441	9.40804668	10.496302	10.0269389	9.3400068	9.33614535	9.80082762
ILMN_29492	FGFR4	10.2733113	6.86294537	7.3741876	7.66506998	7.81822848	7.05119551	7.42664439	7.93693417
ILMN_28273	FGFRL1	9.26064195	10.0769843	8.30818634	8.42022178	9.52599091	9.04788387	7.07266081	8.59600235

Probe	Gene	HCC70	MDA-MB-468	SUM149	MDA-MB-231	BT549	HS578T	MDA-MB-436
ILMN_28534	FGF1	6.78351659	6.71646336	6.79434101	6.85739304	6.62011751	6.81937756	6.75389549
ILMN_28534	FGF1	6.61586341	6.56601635	6.60392578	6.71800664	6.58727185	6.72971375	6.60536727
ILMN_20787	FGF2	6.56738607	6.63109624	6.62339043	6.5714846	6.69628754	6.83062744	7.09829982
ILMN_27066	FGF3	6.63670918	6.70056424	6.6885034	6.69127996	6.69764446	6.67094442	6.69917214
ILMN_12573	FGF4	6.62254043	6.66171409	6.73011865	6.69836239	6.68521158	6.66875393	6.67475344
ILMN_12488	FGF5	6.60298307	6.6799934	6.64135898	6.91662486	6.64665421	6.79263849	6.6145083
ILMN_12829	FGF5	6.6264923	6.59288442	6.6153272	6.95258367	6.63937115	7.09363908	6.61482722
ILMN_28260	FGF6	7.14334748	7.03596069	7.18133173	7.06097158	7.06195011	6.94201809	6.99244061
ILMN_20000	FGF7							
ILMN_1423	FGF8	6.56612988	6.57275819	6.57742642	6.55306006	6.58705729	6.57652443	6.59736199
ILMN_1771	FGF9	6.97693781	6.56872655	6.55312713	6.57074849	6.51781827	6.81501487	6.55543062
ILMN_19131	FGF10	6.64022634	6.60351709	6.61407231	6.63295874	6.64247232	6.62775819	6.59050554
ILMN_22755	FGF16	6.53165677	6.55322155	6.53823175	6.60305448	6.56250486	6.57873416	6.5144131
ILMN_9431	FGF17	6.67434647	6.67806739	6.73738191	6.71179811	6.72717462	6.60535565	6.63000648
ILMN_29689	FGF18	7.16540729	7.01939198	7.23684616	7.21193069	6.97758661	7.03999583	7.09262751
ILMN_8856	FGF18	6.67292712	6.68341249	6.68817101	6.71256559	6.8020021	6.70011121	6.87709443
ILMN_18897	FGF19	6.99850265	6.79774244	6.90324379	6.77742796	6.82983659	6.73206029	6.8491331
ILMN_10954	FGF20	6.54871236	6.60347588	6.62076718	6.72187887	6.70927948	6.67392319	6.65382101
ILMN_9969	FGF21	7.0841353	6.63022714	6.60358006	6.7089026	6.70393635	6.71666137	6.73536467
ILMN_15552	FGF22	6.58595444	6.54421438	6.60920395	6.61161694	6.68892882	6.63176279	6.68907447
ILMN_10473	FGF23	6.86325255	6.69271946	6.75503911	6.80187014	6.68243797	6.73153218	6.75251708
ILMN_7476	FGFR1	6.64573252	6.75888897	6.57852308	6.86610175	6.99153207	6.89180721	6.87927826
ILMN_168227	FGFR2	6.57933726	6.66087188	6.56654639	6.52412333	6.53165045	6.59879475	6.61771931
ILMN_22960	FGFR3	7.15536966	9.0677225	6.67116658	7.81025997	7.56818083	8.01331864	6.93563679
ILMN_22960	FGFR3	6.59781808	6.633695	6.60602189	6.57685674	6.65909985	6.69847851	6.63613333
ILMN_29492	FGFR4	6.88073749	6.7068744	6.98998892	6.64537299	7.70197333	6.96972666	6.76448064
ILMN_28273	FGFRL1	8.06842175	7.54925974	10.0386059	9.77446697	9.06106578	9.69201182	9.74377473

Probe	Gene	HCC1569	MDA-MB-157	SUM225	SUM190	T47D	BT20	HCC1569	MDAMB361	MDAMB453
ILMN_28534	FGF1	6.66578561	6.88517312	7.0859282	6.70827208	6.67502392	6.60671697	7.0778203	7.05412837	6.97245444
ILMN_28534	FGF1	6.58470252	6.8082033	6.80652471	6.67287986	6.62778833	6.49566368	6.89204108	6.88209682	6.91342958
ILMN_20787	FGF2	6.95503433	8.1772105	6.67735571	6.57265347	6.58451702	6.60886562	7.35255011	7.03434264	6.94450568
ILMN_27066	FGF3	6.63376876	6.74007236	6.71200503	6.68993232	6.71067911	6.69316697	6.9948956	7.05576058	7.04363601
ILMN_12573	FGF4	6.68170795	6.65612341	6.62549948	6.63843416	6.66595005	6.58874401	6.95893363	6.86943329	6.96785252
ILMN_12488	FGF5	6.64163555	6.99093021	6.69320755	6.61436759	6.65108499	6.61694639	6.89385777	6.88142492	6.88292594
ILMN_12829	FGF5	6.59919168	7.18957079	6.71273975	6.66347415	6.63180945	6.64978633	6.94694307	7.02758724	6.98448588
ILMN_28260	FGF6	7.00353263	7.03710463	7.05949491	7.07632327	7.06972549	7.02445984	7.30399573	7.25983059	7.37148057
ILMN_20000	FGF7							6.771445	6.86738992	6.85431719
ILMN_1423	FGF8	6.55355089	6.5802823	6.61123233	6.60227193	6.53239956	6.55514674	6.95833204	7.02441017	7.01124942
ILMN_1771	FGF9	7.56000765	6.64400923	7.08068398	7.94778709	6.5352716	6.57942242	8.08372654	6.81509997	6.91118718
ILMN_19131	FGF10	6.6477154	6.62034004	6.62064248	6.58954282	6.64936098	6.6222384	6.91030849	6.91790933	6.93309919
ILMN_22755	FGF16	6.61965641	6.5932616	6.5209844	6.54223613	6.60246057	6.59490166	6.87006174	6.95546149	6.83995305
ILMN_9431	FGF17	6.69061701	6.70277086	6.67900958	6.64995131	6.67276399	6.59741528	6.98325483	7.06461777	7.10134078
ILMN_29689	FGF18	7.17380611	7.04286696	7.13958471	7.0861134	6.95449942	6.99517864	7.39670781	7.45060096	7.42784764
ILMN_8856	FGF18	6.77725758	6.9936608	6.71989076	6.64981418	6.73153095	6.72707903	7.25018934	7.31477304	7.45162841
ILMN_18897	FGF19	6.74649887	6.76097341	6.77213455	6.8770401	6.77405957	6.77232593	7.14179716	7.10525475	7.11035669
ILMN_10954	FGF20	6.5264476	6.56031721	6.63477464	6.60275467	6.58802118	6.61253016	7.00372568	6.98793192	7.15102083
ILMN_9969	FGF21	6.67011504	6.72407864	6.68868249	6.69851534	6.66168574	6.68992855	6.93019029	6.93099559	6.93826875
ILMN_15552	FGF22	6.63976725	6.66178894	6.60067328	6.62786687	6.63863742	6.64558086	6.96790658	7.00931825	6.94228408
ILMN_10473	FGF23	6.78566103	6.7372299	6.76413054	6.76545401	6.77190723	6.64687561	7.05343993	7.01756981	7.11546854
ILMN_7476	FGFR1	6.97085093	7.10977547	6.83699137	6.65876662	6.73200153	6.61478025	7.02744804	7.29855102	6.88414098
ILMN_168227	FGFR2	6.70959414	6.62393653	6.61717432	6.52200204	6.95244614	6.59814944			
ILMN_22960	FGFR3	9.3152741	7.11145535	9.55050409	6.72955431	10.2930192	8.90671204	9.33274983	10.5921012	7.46215242
ILMN_22960	FGFR3	6.64346445	6.67935656	6.61884747	6.57967741	6.66735094	6.70446037	7.03894868	7.01702105	7.02621375
ILMN_29492	FGFR4	8.03146262	6.63985607	7.78422671	6.93173969	6.94235328	6.80709278	7.5387744	9.12624012	11.1374481
ILMN_28273	FGFRL1	9.32536	10.8280488	7.2213045	8.3028402	8.77044252	8.35784695	8.94475046	8.30617732	8.0543154

Supplementary table 3. Tumour features associated with nuclear FGF2 expression, in the absence of cytoplasmic expression, compared to cancers with no FGF2 expression in an invasive breast cancer tissue microarray.

	FGF2 positive	FGF2 negative	p value
n	19	152	
Pathology			
IDC	14	109	0.86*
ILC	4	38	
Other	1	5	
Median tumour size (mm)	15	21	0.06+
Histological grade			
1	2	21	0.18*
2	10	46	
3	7	80	
Vascular invasion	68% (13/19)	70% (106/152)	1
Axillary node positive	57% (11/19)	68% (103/152)	0.3
ER positive	89% (17/19)	87% (132/152)	1
PR positive	79% (15/19)	78% (119/152)	1
HER2 positive	5% (1/19)	13% (20/152)	0.47
EGFR	0% (0/19)	5% (7/152)	1
ck5/6	0% (0/19)	5% (8/152)	0.6
ck14	5% (1/19)	4% (6/152)	0.57
Any basal marker	5% (1/19)	9% (13/152)	1
Tumour subtype			
Luminal	16	116	0.7*
HER2	1	21	
TN Basal-like	1	8	
TN non basal	1	7	

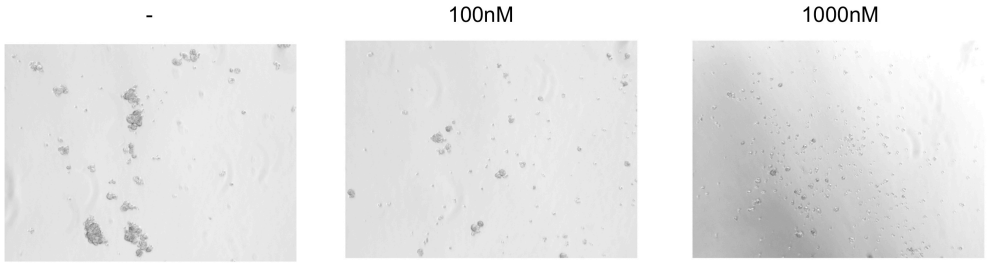
Supplementary table 4. FGF signalling aberrations in triple negative breast cancer cell lines

Cell line	PD173074 Sensitivity	Abberation
BT20	N	
BT549	Y	FGF2 expression
CAL120	Y	*FGFR1* amplification and FGF2 expression
CAL51	Y	FGF2 expression
HCC1143	Y	*FGFR2* mutation (R203C uncertain pathogenesis)
HCC1187	N	
HCC1500	N/A	
HCC38	N/A	FGF2 expression
HCC70	N	
HMT3552	N	FGF2 expression
HS578T	Y	FGF2 expression
MDAMB157	Y	FGF2 expression
MDAMB213	N	
MDAMB436	N	FGF2 expression
MDAMB468	N	
MFM223	Y	*FGFR2* amplification
SUM149	N	
SUM159	N/A	FGF2 expression
SUM1315M	N/A	FGF2 expression
SUM225	N/A	
SUM52PE	Y	*FGFR2* amplification

PD173074 sensitivity – Y significant reduction in growth ($p<0.01$), N No change, N/A not tested. FGF2 expression defined as level of FGF2 mRNA expression that results in detectable FGF2 protein by western blotting (Figure 3B).

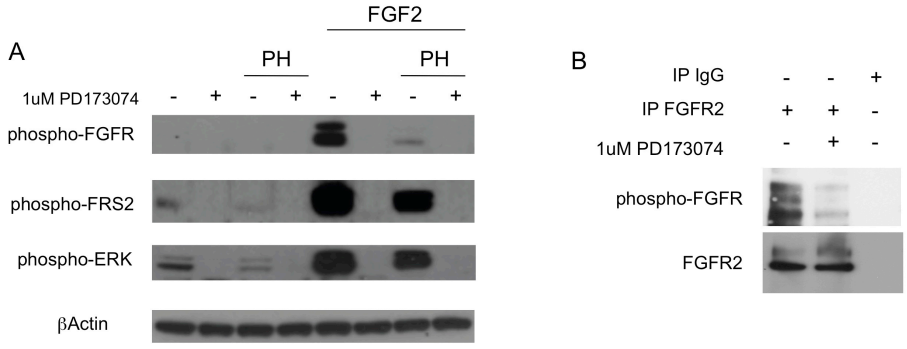
Supplementary Figures

Supplementary Figure 1. CAL51 are sensitive to PD173074 in soft agar assay at sub-micromolar concentrations.



Repeat soft agar assays of CAL51 cells grown for 2 weeks continuously in the presence of indicated concentration of PD173074, with decreased growth at 100nM and 1000 nM.

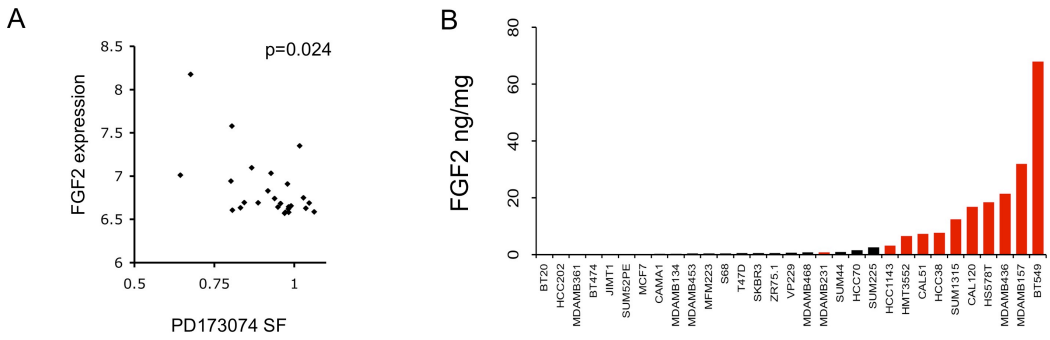
Supplementary Figure 2. Inhibition of FGFR signalling in CAL120 and CAL51 by PD173074



A. Lysates of CAL120 cells growing on polyHEMA treated, on untreated, plates were treated for 1 hour with 1μM PD173074 (+) or no treatment (-). In addition, cells were treated with FGF2 1ng/ml for 5 minutes, or no treatment as indicated, prior to lysis. Both FGFR Tyr653/654 (residue location in FGFR1 IIIc) and FRS2 Tyr196 phosphorylation are inhibited by PD173074. PH - polyHEMA

B. Lysates from CAL51 cells growing in polyHEMA treated plates treated for 1 hour with 1μM PD173074 (+) or no treatment (-). Cell were treated with 1ng/ml FGF2 for 5mins prior to lysis. Lystates were immunoprecipitated (IP) with FGFR2 antibody, and blotted for phospho-FGFR Tyr653/654 and FGFR2. For an IP control lysates from CAL51 cells growing on polyHEMA coated plates treated with FGF2 were immunoprecipitated with normal goat IgG (IP IgG).

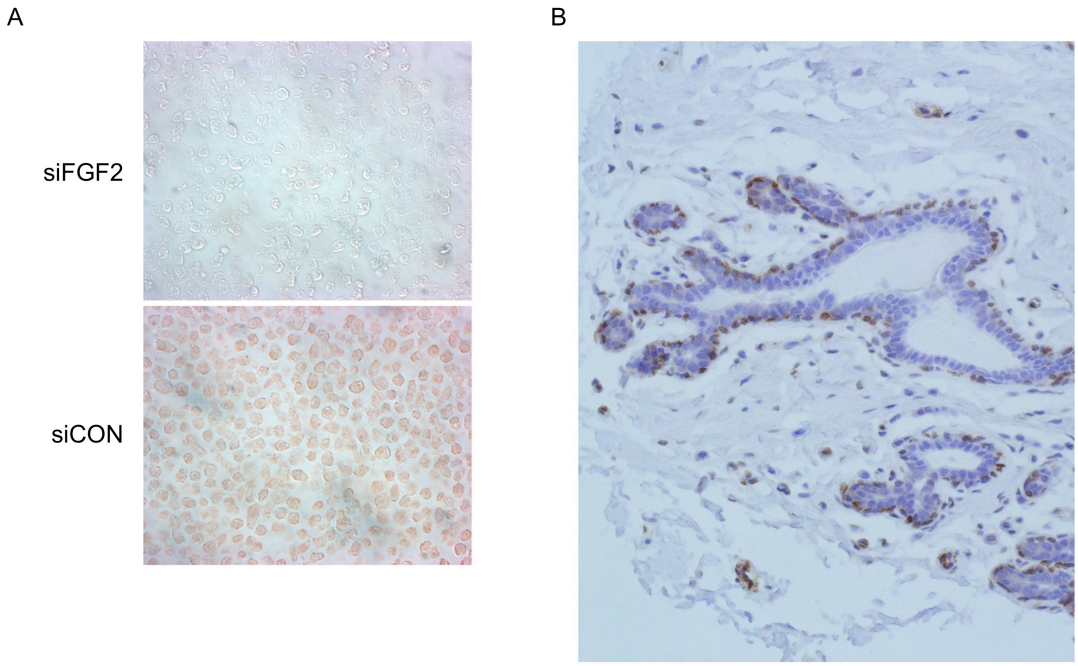
Supplementary Figure 3. FGF2 expression in Basal cell lines



A. Correlation of *FGF2* mRNA expression assessed by whole genome gene expression arrays with PD173074 survival fraction, in 29 breast cancer cell lines excluding cancers with known *FGFR2* amplification. Spearman correlation coefficient -0.43, p=0.024 (Correlation including *FGFR2* amplified cancer cell lines -0.41, p=0.027)

B. FGF2 quantification by ELISA in cell lysates assessed as ng FGF2 protein per mg of total cellular protein. Red indicated Basal-B like cell lines, which express more FGF2 than comparator cell lines (P<0.0001 Mann Whtiney U Test).

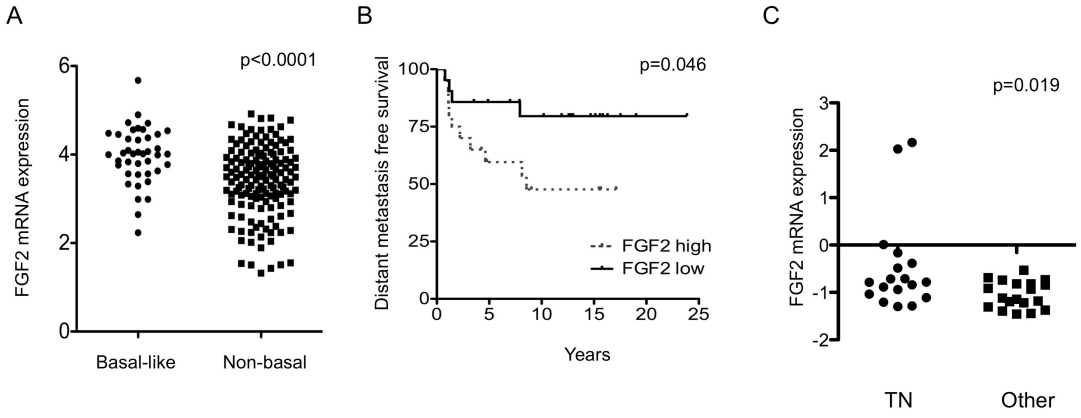
Supplementary Figure 4. Validation of FGF2 immunohistochemistry and normal tissue staining.



A. Validation of FGF2 immunohistochemistry. Hela cells transfected with siFGF2 or siCON, were pelleted, fixed in formalin and embedded in paraffin, prior to FGF2 immunohistochemistry.

B. Representative image of FGF2 immunohistochemistry of a normal breast duct demonstrating FGF2 expression in the myoepithelial cells.

Supplementary Figure 5. *FGF2* mRNA expression assessed in external whole genome expression array data set.

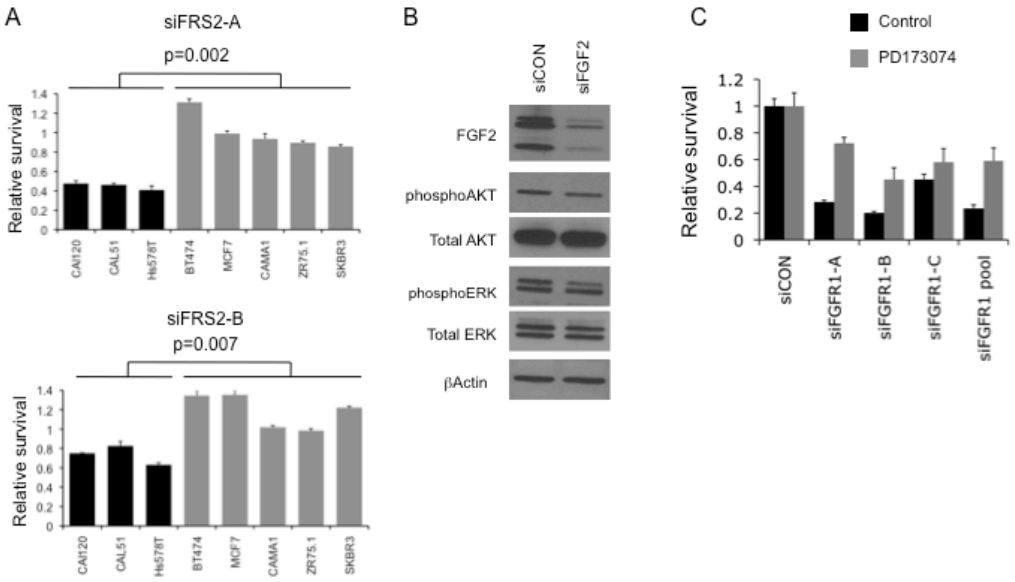


A. Assessment of *FGF2* mRNA expression (mean 204421_s_at and 204422_s_at, arbitrary units) in 188 breast cancers in previously published gene expression array data (1), with basal-like tumours defined by PAM50 (i.e. Parker et al (2) single sample predictor) as in Weigelt et al (3). FGF2 mRNA expression is expressed at significantly higher levels in basal-like breast cancers (p=0.001 Mann-Whitney U Test).

B. Kaplan Meier survival analysis of the 41 basal-like breast cancer from part A, comparing cancers with high FGF2 expression (FGF2 expression > median) and low FGF2 expression (FGF2 expression ≤ median) (p=0.046 Log-rank test).

C. FGF2 mRNA expression in Richardson et al (4) in 37 breast cancers defined according to triple negative status (TN) or other subtype (other). FGF2 is significantly over-expressed in TN breast cancer (p=0.019 Mann Whitney U Test). FGF2 data was extracted from www.oncomine.org and expressed as log2 median-centered intensity.

Supplementary Figure 6. Basal cell lines are dependent on FGFR signalling

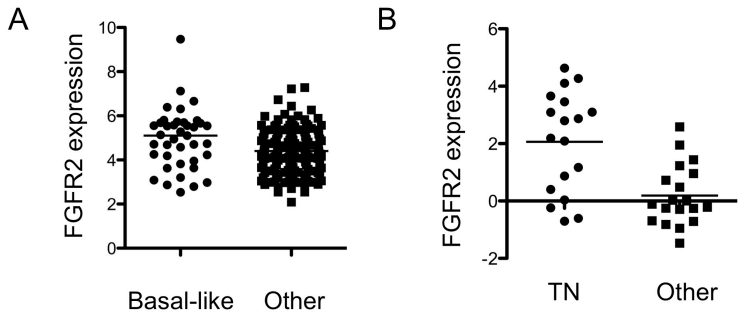


A. Indicated cell lines were transfected with siCON and two individual siRNA targeting FRS2 (left siFRS2-A and right siFRS2-B) and survival was assessed 5 days post transfection. Survival with siRNA targeting FRS2 was expressed relative to siCON. The three basal-like cell lines (CAL120, CAL51, and Hs578T - black) show significantly reduced survival with FRS2 knockdown compared to cell lines expressing ER and or HER2 (FRS2-A p=0.002 and FRS2-B p=0.007, Student's T-test). A third siRNA siFRS2-C similarly reduced the survival of basal-like cell lines to a greater extent than all control cell lines (p=0.001), but reduced the growth of all cell lines compared to siCON presumably due to additional off-target effects (data not shown).

B. CAL120 cells were transfected with siCON and siFGF2, lysates were made 72 hours post transfection and subject to western blotting with indicated antibodies

C. CAL120 cells were transfected with siCON, three individual siRNA targeting FGFR1 (siFGFR1 A-C), and FGFR1 SMARTpool (siFGFR1). Two days post transfection cells were exposed to PD173074 1μM, or control, and survival was assessed 5 days post transfection and expressed relative to the relevant siCON. CAL120 cells are less sensitive to FGFR1 silencing in the presence of PD173074.

Supplementary Figure 7. FGFR2 expression assessed in external whole genome expression array data set.



A. Assessment of FGFR2 mRNA expression (arbitrary units) in 188 breast cancers in previously published gene expression array data (1), with basal-like tumours defined by PAM50 (3). *FGFR2* mRNA is expressed at significantly higher levels in basal-like breast cancers (p=0.008 Mann-Whitney U Test).

B. *FGFR2* mRNA expression in Richardson et al (4) in 37 breast cancers defined according to triple negative status (TN) or other subtype (other). FGFR2 is significantly over-expressed in TN breast cancer (p=0.0013 Mann Whitney U Test). *FGFR2* (211301_s_at) data extracted from www.oncomine.org and expressed as log2 median-centered intensity.

Supplementary methods

Materials

Antibodies used were phosphorylated FGFR-Tyr653/654 (Cell signalling, 3471), FRS2-Tyr196 (Cell signalling, 3864), FGFR2 (Santa Cruz, sc-122). siRNA were siGenome individual siRNA (siFRS2-A-C D-006440-03/05/18), siFGFR1 siGenome individual siRNA (siFGFR1 A-C D003131-09/22/23) and siFGFR1 SMARTpool (M003131-03).

Array Comparative genomic hybridisation

ArrayCGH was performed on the 32K BAC re-array collection (CHORI) tiling path aCGH platform and analysed as previously described (5). Data will be made publicly available at http://rock.icr.ac.uk/.

Microarray gene expression

Gene expression profiling was performed using the Illumina human WG6 version 2 expression array according to the manufacturer's protocol, and analysed as previously described (6). Data will be made publicly available at ArrayExpress.

Analysis of FGF2 and FGFR2 mRNA expression in external data sets

For analysis of FGF2 mRNA expression data we analysed the data set of Desmedt et al (1) identifying basal-like breast cancers by PAM50 (2), which we have previously shown robustly identifies basal-like from other types of breast cancers (3). FGF2 expression was assessed as the mean of 204421_s_at and 204422_s_at. FGF2 expression was at the limit of detection of the Affymetrix probes used in this analysis. FGFR2 expression was assessed as the mean of 203639_s_at, 208228_s_at, and 211401_s_at. Analysis between groups was with the Mann-Whitney U test.

Supplementary references

1. Desmedt C, Piette F, Loi S, et al. Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. Clin Cancer Res 2007;13:3207-14.

2. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160-7.

3. Weigelt B, Mackay A, A'Hern R, et al. Breast cancer molecular profiling with single sample predictors: a retrospective analysis. Lancet Oncol;11:339-49.

4. Richardson AL, Wang ZC, De Nicolo A, et al. X chromosomal abnormalities in basal-like human breast cancer. Cancer Cell 2006;9:121-32.

5. Lacroix-Triki M, Suarez PH, Mackay A, et al. Mucinous carcinoma of the breast is genomically distinct from invasive ductal carcinomas of no special type. J Pathol 2010.

6. Natrajan R, Weigelt B, Mackay A, et al. An integrative genomic and transcriptomic analysis reveals molecular pathways and networks regulated by copy number aberrations in basal-like, HER2 and luminal cancers. Breast Cancer Res Treat 2010;121:575-89.